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(21) International Application Number: PCT/US89/05782 (22) International Filing Date: 15 December 1989 (15.12.89) (30) Priority data: 284,876 19 December 1988 (19.12.88) US (71) Applicant: THE DOW CHEMICAL COMPANY [US/ US]; 2030 Dow Center, Abbott Road, Midland, MI 48640 (US). (72) Inventors: SIMON, Jaime ; Route 1, Box 120-G, Angleton, TX 77515 (US). WILSON, David, A. ; 229 San Saba, Richwood, TX 77531 (US). GARLICH, Joseph, R. ; 301 Southern Oaks, Lake Jackson, TX 77566 (US). TROUT- NER, David, E. ; 402 Edgewood Avenue, Columbia, MO 65201 (US).	(74) Agent: KIMBLE, Karen, L.; The Dow Chemical Com- pany, P.O. Box 1967, Midland, MI 48641-1967 (US). (81) Designated States: AT (European patent), AU, BE (Euro- pean patent), BR, CH (European patent), DE (European patent), DK, ES (European patent), FI, FR (European patent), GB (European patent), HU, IT (European pa- tent), JP, KR, LU (European patent), NL (European pa- tent), NO, SE (European patent). Published With international search report With amended claims	
(54) Title: MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE <div style="text-align: center; margin: 20px 0;"> $\left(\begin{array}{c} \text{X} \\ \\ -\text{C}- \\ \\ \text{Y} \end{array} \right)_n$ </div>		
(57) Abstract <p>Particle emitting radionuclides, e.g. Samarium-153, have been complexed with certain macrocyclic aminophosphonic acids wherein the nitrogen and phosphorus are interconnected by an alkylene group or substituted alkylene group. A composition is now disclosed which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene radical of formula (I), wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the proviso that when n > 1, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175.</p>		

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MACROCYCLIC AMINOPHOSPHONIC ACID COMPLEXES, THEIR PREPARATION, FORMULATIONS AND USE

The present invention concerns macrocyclic aminophosphonic acid complexes for the treatment of cancer, especially the treatment of calcific tumors and for the relief of bone pain, the method of treatment of calcific tumors, and compositions and formulations having as their active ingredient a radionuclide complexed with a macrocyclic aminophosphonic acid, and the process for preparing the macrocyclic aminophosphonic acid complexes.

The development of bone metastasis is a common and often catastrophic event for a cancer patient. The pain, pathological fractures, frequent neurological deficits and forced immobility caused by these metastatic lesions significantly decrease the quality of life for the cancer patient. The number of patients that contract metastatic disease is large since nearly 50 percent of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastasis. Bone metastasis are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix and other tumors, but collectively, these represent less than 20 percent of patients who develop bone metastasis. Metastatic bone cancer is rarely life threatening and

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occasionally patients live for years following the discovery of the bone lesions. Initially, treatment goals center on relieving pain, thus reducing requirements for narcotic medication and increasing
5 ambulation. Clearly, it is hoped that some of the cancers can be cured.

The use of radionuclides for treatment of cancer metastatic to the bone dates back to the early
10 1950's. It has been proposed to inject a radioactive particle-emitting nuclide in a suitable form for the treatment of calcific lesions. It is desirable that such nuclides be concentrated in the area of the bone lesion with minimal amounts reaching the soft tissue and
15 normal bone. Radioactive phosphorus (P-32 and P-33) compounds have been proposed, but the nuclear and biolocalization properties limit the use of these compounds. [See for example, Kaplan, E., et al., *Journal of Nuclear Medicine* 1(1), 1 (1960) and U.S. Patent
20 3,965,254.]

Another attempt to treat bone cancer has been made using phosphorus compounds containing a boron
25 residue. The compounds were injected into the body (intravenously) and accumulated in the skeletal system. The treatment area was then irradiated with neutrons in order to activate the boron and give a therapeutic radiation dose. (See U.S. Patent
30 4,399,817).

The use of radionuclides for calcific tumor therapy is discussed in published European patent application 176,288 where the use of Sm-153, Gd-159,
35 Ho-166, Lu-177 or Yb-175 complexed with certain ligands selected from ethylenediaminetetraacetic acid

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(EDTA) or hydroxyethylethylenediaminetriacetic acid (HEEDTA) is disclosed.

5 In the above mentioned procedures, it is not possible to give therapeutic doses to the tumor without substantial damage to normal tissues. In many cases, especially for metastatic bone lesions, the tumor has spread throughout the skeletal system and amputation or external beam irradiation is not
10 practical. (See Seminars in Nuclear Medicine, Vol. IX, No. 2, April, 1979).

The use of Re-186 complexed with a diphosphonate has also been proposed. [Mathieu, L. et
15 al., *Int.J. Applied Rad. & Isotopes* 30, 725-727 (1979); Weinenger, J., Ketring, A. R., et al., *Journal of Nuclear Medicine* 24(5), 125 (1983)]. However, the preparation and purification needed for this complex limits its
20 utility and wide application.

Strontium-89 has also been proposed for patients with metastatic bone lesions. However, the long half-life (50.4 days), high blood levels and low
25 lesion to normal bone ratios limit the utility. [See Firusian, N., Mellin, P., Schmidt, C. G., *The Journal of Urology* 116, 764 (1976); Schmidt, C. G., Firusian, N., *Int.J. Clin. Pharmacol.* 93, 199-205, (1974).]

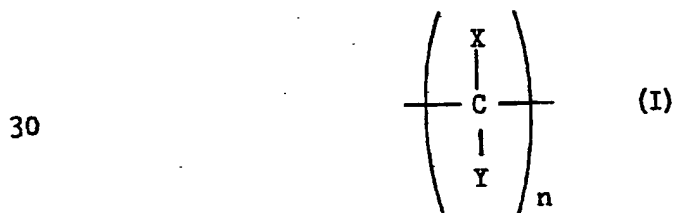
A palliative treatment of bone metastasis has
30 been reported which employed I-131 labeled α -amino-(3-iodo-4-hydroxybenzylidene)diphosphonate [Eisenhut, M., *Journal of Nuclear Medicine* 25(12), 1356-1361 (1984)]. The use of radioactive iodine as a therapeutic
35 radionuclide is less than desirable due to the well known tendency of iodine to localize in the thyroid.

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Eisenhut lists iodide as one of the possible metabolites of this compound.

Surprisingly, the present invention overcomes many of the above noted problems. The present invention concerns at least one composition having a radionuclide complexed with a macrocyclic aminophosphonic acid, such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or its physiologically acceptable salt, which composition causes minimal damage to normal tissue when administered in the method of the invention. Surprisingly, the present complex is more effective at a lower ligand to metal molar ratio than has been known previously in the art.

Particularly, this invention concerns a composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula

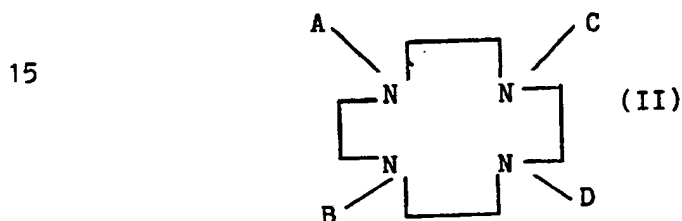


wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable

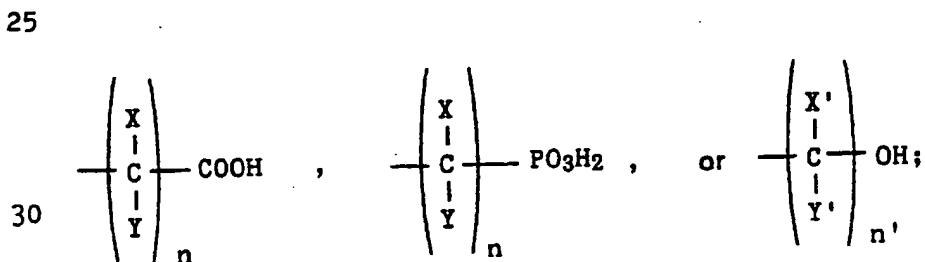
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salts of the acid radicals; and n is 1-3, with the proviso that when $n > 1$, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, and

wherein the resulting composition is therapeutically effective. Particularly preferred are macrocyclic moieties of Formula (I) where X and Y are hydrogen and n is 1. Especially preferred are a certain macrocyclic aminophosphonic acid of the structure



20 wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula



35 and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined before; X' and Y' are independently hydrogen, methyl or ethyl

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radicals; n' is 2 or 3, with the proviso that at least two of said nitrogen substituents is a phosphorus-containing group. The preferred macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclo-
5 dodecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP). The composition can be administered as a formulation with suitable pharmaceutically acceptable carriers. The present invention includes the use of the complex, composition or formulation described
10 herein in combination with one or more other agents, drugs, treatments and/or radiation sources which assist in therapy of calcific tumors or relief of bone pain .

Certain compositions containing these
15 complexes have been found useful for therapy of calcific tumors in animals. The administration of the therapeutic compositions can be palliative to the animal, for example by alleviating pain and/or
20 inhibiting tumor growth and/or causing regression of tumors and/or destroying the tumors. As will be more fully discussed later, the properties of the radionuclide, of the macrocyclic aminophosphonic acid and of the complex formed therefrom are important
25 considerations in determining the effectiveness of any particular composition employed for such treatment.

In addition, the present invention also includes formulations having at least one of the
30 radionuclide(s) complexed with at least one of the macrocyclic aminophosphonic acids as defined above, especially those macrocyclic aminophosphonic acids of Formula (II), and a pharmaceutically acceptable carrier, excipient or vehicle therefor. The methods for
35 preparing such formulations are well known. The formulations are sterile and may be in the form of a

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suspension, injectable solution or other suitable pharmaceutically acceptable formulations.

Pharmaceutically acceptable suspending media, with or without adjuvants, may be used. The sterile

5 compositions are suitable for administration to an animal wherein the composition is defined as before and has the radionuclide in dosage form present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal, preferably at least 0.2 mCi per kilogram
10 of body weight of said animal..

Particle-emitting radionuclides employed in the compositions of the invention are capable of delivering a high enough localized ionization density
15 to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors, and/or destroy the tumor and are capable of forming complexes with the macrocyclic aminophosphonic acid ligands described herein. The radionuclides found to be useful in the
20 practice of the invention are Samarium-153 (Sm-153), Holmium-166 (Ho-166), Ytterbium-175 (Yb-175), Lutetium-177 (Lu-177), Yttrium-90 (Y-90) and Gadolinium-159 (Gd-159).

25 For the purpose of convenience, the compositions having a radionuclide-macrocyclic aminophosphonic acid complex of the present invention will frequently be referred to herein as "radionuclide
30 compositions" or "compositions" and the macrocyclic aminophosphonic acid derivative referred to as the "ligand" or "chelant".

As used herein, the term "animals" means warm
35 blooded mammals, including humans, and is meant to

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encompass animals in need of treatment for calcific tumors or in need of relief of bone pain.

5 The term "calcific tumors" includes primary tumors, where the skeletal system is the first site of involvement, invasive tumors where the primary tumor invades the skeletal system or other tissue tumors which calcify, and metastatic bone cancer where the neoplasm spreads from other primary sites, e.g.
10 prostate and breast, into the skeletal system.

 For the purpose of the present invention, the complexes described herein and physiologically acceptable salts thereof are considered equivalent in
15 the therapeutically effective compositions. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand or ligands employed and which will not cause a significant
20 adverse physiological effect when administered to an animal at dosages consistent with good pharmacological practice; some examples of such practice are described herein. Suitable bases include, for example, the
25 alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary
30 amines and the like. Physiologically acceptable salts may be prepared by treating the macrocyclic aminophosphonic acid as defined above, especially those of Formula (II), with an appropriate base.

35 The formulations of the present invention are in the solid or liquid form containing the active

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radionuclide complexed with the ligand. These formulations may be in kit form such that the two components are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier. Additionally, for stability and other factors, if the formulations are complexed with the radionuclide prior to shipment to the ultimate user, the formulation having the complex and a buffer present are frozen in a kit form, and which frozen formulation is later thawed prior to use.

Injectable compositions of the present invention may be either in suspension or solution form. In the preparation of suitable formulations it will be recognized that, in general, the water solubility of the salt is greater than the free acid. In solution form the complex (or when desired the separate components) is dissolved in a pharmaceutically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, water, aqueous alcohols, glycols, and phosphonate or carbonate esters. Such aqueous solutions contain no more than 50 percent of the organic solvent by volume.

Injectable suspensions as compositions of the present invention require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethylcellulose. Suitable physiologically acceptable adjuvants, if necessary to keep the complex in suspension, may be chosen from among thickeners such as carboxymethyl-

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cellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters. Many substances which effect the hydrophobicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoams, sorbitol, and sugars are all useful suspending agents.

Complexes employed in the compositions or formulations of the present invention must fit certain criteria insofar as possible as discussed below.

One criteria concerns the selection of the radionuclide. While the properties of the radionuclide are important, the overall properties of the composition containing the radionuclide-macrocylic aminophosphonic acid complex is the determining factor. The disadvantages of any one property may be overcome by the superiority of one or more of the properties of either ligand or radionuclide and their combination, as employed in the composition must be considered in toto.

There is a need for compositions possessing the following criteria by which it is possible to deliver therapeutic radiation doses to calcific tumors with minimal doses to soft tissue. For example, the radionuclide must be delivered preferentially to the bone rather than to soft tissue. Most particularly, uptake of the radionuclide in either liver or blood is undesirable. Additionally, the radionuclide should be

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cleared rapidly from non-osseous tissue to avoid unnecessary damage to such tissues, e.g., it should clear rapidly from the blood.

5 The proposed use for the compositions and formulations of this invention is the therapeutic treatment of calcific tumors in animals. As used herein, the term "calcific tumors" includes primary tumors where the skeletal system is the first site of
10 involvement, or other tissue tumors which calcify, or metastatic bone cancer where the neoplasm spreads from other primary sites, such as prostate and breast, into the skeletal system. This invention provides a means of alleviating pain and/or reducing the size of,
15 and/or inhibiting the growth and/or spread of, or causing regression of and/or destroying the calcific tumors by delivering a therapeutic radiation dose.

20 The composition or formulation may be administered as a single dose or as multiple doses over a longer period of time. Delivery of the radionuclide to the tumor must be in sufficient amounts to provide the benefits referred to above.

25 The "effective amount" or "therapeutically effective amount" of radionuclide composition to be administered to treat calcific tumors will vary according to factors such as the age, weight and health
30 of the patient, the calcific tumor being treated, the treatment regimen selected as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the
35 emissions will also be a factor in determining the amount of activity necessary. The compositions of this

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invention may also be employed at doses which are useful but not therapeutic.

5 A suitable dose of the composition or formulation of this invention for use in this invention is at least about 0.02 mCi per Kg of body weight. A "therapeutically effective dose" of the composition or formulation of this invention for use in this invention is at least about 0.2 mCi per Kg of body weight.

10 The effective amount used to treat calcific tumors will typically be administered, generally by administration into the bloodstream, in a single dose or multipule doses. The amounts to be administered to
15 achieve such treatment are readily determined by one skilled in the art employing standard procedures.

The radionuclide and ligand may be combined under any conditions which allow the two to form a
20 complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is all that is required. The complex formed is by a chemical bond and results in a relatively stable radionuclide composition, e.g. stable to the
25 disassociation of the radionuclide from the ligand.

The macrocyclic aminophosphonic acid complexes when administered at a ligand to metal molar ratio of at least about 1:1, preferably from 1:1 to 3:1, more
30 preferably from 1:1 to 1.5:1, give biodistributions that are consistent with excellent skeletal agents. By contrast, certain other aminophosphonic acid complexes result in some localization in soft tissue (e.g. liver)
35 if excess amounts of ligand are not used. A large excess of ligand is undesirable since uncomplexed ligand

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may be toxic to the patient or may result in cardiac arrest or hypocalcemic convulsions. In addition, the macrocyclic aminophosphonic acid ligands are useful when large amounts of metal are required (i.e. for metals that have a low specific activity). In this case, the macrocyclic aminophosphonic acid ligands have the ability to deposit larger amounts of activity in the bone than is possible when using non-cyclic aminophosphonic acid ligands.

10

A preferred embodiment of the present invention is a therapeutically effective composition or formulation containing complexes of at least one radionuclide of Gd-159, Ho-166, Lu-177, Sm-153, Y-90 and Yb-175 with DOTMP or a physiologically acceptable salt(s) thereof.

15

Combinations of the various above noted radionuclides can be administered for the therapeutic treatment of calcific tumors. The combinations can be complexed as herein described by complexing them simultaneously, mixing two separately complexed radionuclides, or administering two different complexed radionuclides sequentially. It may be possible to achieve the same beneficial results of high delivery of the radionuclide to the area of the tumor, but with little soft tissue damage, by administering the ligand and the radionuclide in a manner which allows formation of the radionuclide-chelant complex *in situ* such as by simultaneous or near simultaneous administration of the radionuclide and an appropriate amount of ligand or by the administration of ligand and a radionuclide complexed with a weaker ligand, i.e., one which undergoes ligand exchange with the ligands of this invention, such that the desired

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radionuclide-chelant complex is formed via ligand exchange *insitu*. The composition or formulation may be administered as a single dose or as multiple doses over a longer period of time.

5

Aminophosphonic acids can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive amine hydrogen with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof. The amine precursor (1,4,7,10-tetraazacyclododecane) employed in making the macrocyclic aminophosphonic acids is a commercially available material.

15

Methods for carboxyalkylating to give amine derivatives containing a carboxyalkyl group are well known (U.S. 3,726,912) as are the methods which give alkyl phosphonic and hydroxyalkyl (U.S. 3,398,198) substituents on the amine nitrogens.

20

Radionuclides can be produced in several ways. In a nuclear reactor, a nuclide is bombarded with neutrons to obtain a radionuclide, e.g.

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Another process for obtaining radionuclides is by bombarding nuclides with linear accelerator or cyclotron-produced particles. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The process for obtaining

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the radionuclide is not critical to the present invention.

For example, to irradiate Sm_2O_3 for production of Sm-153, the desired amount of target was first weighed into a quartz vial, the vial was flame sealed under vacuum and welded into an aluminum can. The can was irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial was removed and transferred to a glove box, crushed into a glass vial which was then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1 to 4M HCl was then added to the vial via syringe to dissolve the Sm_2O_3 . Once dissolved, the solution was diluted to the appropriate volume by addition of water. The solution was removed from the original dissolution vial which contains shards of the crushed quartz vial and transferred via syringe to a clean glass serum vial. This solution was then used for complex preparation. Similar procedures can be used to prepare Lu-177, Yb-175, Gd-159, Y-90 and Ho-166.

The invention described herein provides a means of delivering a therapeutic amount of radioactivity to calcific tumors. However, it may also be desirable to administer a "sub-therapeutic" amount (i.e. "useful amount") to determine the fate of the radionuclide using a scintillation camera prior to administering a therapeutic dose. Therapeutic doses will be administered in sufficient amounts to alleviate pain and/or inhibit tumor growth and/or cause regression of tumors and/or kill the tumor. Amounts of radionuclide needed to provide the desired therapeutic dose will be determined experimentally and

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optimized for each particular composition. The amount of radioactivity required to deliver a therapeutic dose will vary with the individual composition employed. For example, less activity will be needed for radionuclides with longer half-lives. The energy of the emissions will also be a factor in determining the amount of activity necessary. The composition to be administered may be given in a single treatment or fractionated into several portions and administered at different times. Administering the composition in fractionated doses may make it possible to minimize damage to non-target tissue. Such multiple dose administration may be more effective.

The compositions of the present invention may be used in conjunction with other active agents and/or ingredients that enhance the therapeutic effectiveness of the compositions and/or facilitate easier administration of the compositions.

Studies to determine the qualitative biodistribution of the various radionuclides were conducted by injecting the compositions into rats and obtaining the gamma ray images of the entire animal at various times up to two hours after injection.

Quantitative biodistributions were obtained by injecting 50-100 microliters of the composition into the tail vein of unanesthetized male Sprague Dawley rats. The rats were then placed in cages lined with absorbent paper in order to collect all urine excreted prior to sacrifice. After a given period of time, the rats were sacrificed by cervical dislocation and the various tissues dissected. The samples were then rinsed with saline, blotted dry on absorbent paper and

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weighed. The radioactivity in the samples was measured with a NaI scintillation counter.

5 The following examples are included to aid in the understanding of the invention but are not to be construed as limiting the invention.

Preparation of Starting Materials

10 Example A: Preparation of DOTMP

In a 100-mL three necked round-bottomed flask equipped with a thermometer, reflux condenser, and heating mantle was added 3.48 g (20.2 mmole) of 1,4,7,10-tetraazacyclododecane and 14 ml of water. This
15 solution was treated with 17.2 mL of concentrated HCl and 7.2 g of H_3PO_3 (87.8 mmole) and heated to 105°C. The refluxing suspension was stirred vigorously and treated dropwise with 13 g (160.2 mmole) of formaldehyde
20 (37 wt percent in water) over a one hour period. At the end of this time the reaction was heated at reflux an additional 2 hours after which the heat was removed and the reaction solution allowed to cool and set at room temperature for 62.5 hours. The reaction solution was
25 then concentrated *in vacuo* at 40°C to a viscous reddish brown semisolid. A 30 mL portion of water was added to the semisolid which started to dissolve but then began to solidify. The whole suspension was then poured into 400 mL of acetone with vigorously stirring. The
30 resulting off-white precipitate was vacuum filtered and dried overnight to give 10.69 g (97 percent yield) of crude DOTMP. A 2.0 g (3.65 mmole) sample of the crude DOTMP was dissolved in 2 mL of water by the addition of
35 700 μ L of concentrated ammonium hydroxide (10.0 mmole) in 100 μ L portions to give a solution at pH of 2-3.

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This solution was then added all at once to 4.5 mL of 3N HCl (13.5 mmole), mixed well, and allowed to set. Within one hour small squarish crystals had begun to form on the sides of the glass below the surface of the liquid. The crystal growth was allowed to continue undisturbed for an additional 111 hours after which time the crystals were gently bumped off of the vessel walls, filtered, washed with 3 mL portions of water, four times, and air dried to constant weight to give 1.19 g (60 percent yield) of white crystalline solid DOTMP.

Example B: Preparation of DOTMP

A 250 mL three-necked, round-bottomed flask was loaded with 6.96 g (0.04 moles) of 1,4,7,10-tetraazacyclododecane. To this flask was added 14.5 g (0.177 moles) of phosphorous acid, 30 mL of deionized water and 28 mL of concentrated hydrochloric acid (0.336 moles).

The flask was attached to a reflux condenser and fitted with a stir bar, and a thermometer adapted with a thermowatch controller. A separate solution of 26.0 g (0.32 moles) of aqueous 37 percent formaldehyde solution was added to a 100 mL addition funnel and attached to the flask. The flask was brought to reflux temperature (about 105°C) with vigorous stirring. The formaldehyde solution was added dropwise over a 30-40 minute interval. The solution was heated and stirred for an additional three hours then cooled slowly to ambient temperature.

The reaction solution was transferred to a 500 mL round-bottomed flask and attached to a rotary evaporation apparatus. The solution was taken down to a

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viscous, amber semi-solid (note - temperature never exceeded 40°C). This semi-solid was treated with approximately 300 mL of HPLC grade acetone producing a light brown, sticky viscous oil. This oil was dissolved in 22 mL of water and added slowly with vigorous stirring to 1L of acetone. The acetone was decanted and the light colored oil dried under vacuum to give 16.6 g (76 percent yield) of crude DOTMP. To 13.1 g of this crude DOTMP was added 39.3 g of deionized water along with a seed crystal and the solution allowed to stand overnight. The resulting precipitate was vacuum filtered, washed with cold water, and dried under vacuum to give 4.75 g of DOTMP (36 percent yield).

A further purification was performed by dissolving 3.0 g (5.47 mmole) of DOTMP from above in 3 mL of water by the addition of 2.2 mL (31.5 mmole) of concentrated ammonium hydroxide. This solution was made acidic by the addition of 2.4 mL (28.8 mmole) of concentrated HCl at which time a white solid precipitated. This precipitate was vacuum filtered and dried to give 2.42 g (81 percent yield) of purified DOTMP characterized by a singlet at 11.5 ppm (relative to 85 percent H_3PO_4) in the ^{31}P decoupled NMR spectrum.

Example C: Preparation of Sm-153

Sm-153 can be produced in a reactor such as the University of Missouri Research Reactor. Sm-153 is produced by irradiating 99.06 percent enriched $^{152}\text{Sm}_2\text{O}_3$ in the first row reflector at a neutron flux of 8×10^{13} neutron/cm².sec. Irradiations were generally carried out for 50 to 60 hours, yielding a Sm-153 specific activity of 1000-1300 Ci/g.

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To irradiate Sm_2O_3 for production of Sm-153, the desired amount of target is first weighed into a quartz vial, the vial flame sealed under vacuum and welded into an aluminum can. The can is irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial is removed and transferred to a glove box, opened into a glass vial which is then sealed. An appropriate amount of a solution of hydrochloric acid is then added to the vial via syringe in order to dissolve the Sm_2O_3 . Once the Sm_2O_3 is dissolved, the Samarium solution is diluted to the appropriate volume by addition of water. The solution is removed from the original dissolution vial which contains the chards of the quartz irradiation vial, and transferred via syringe to a clean glass serum vial.

Example D: Preparation of Ho-166

Holmium-166 is prepared by weighing 0.5-1.0 mg of Ho_2O_3 into a quartz vial. The vial is sealed and placed in an aluminum can which is welded shut. The sample is irradiated (usually for about 24-72 hours) in the reactor (first row reflector, neutron flux of 8×10^{13} neutron/cm².sec). After irradiation, the vial is opened and the oxide is dissolved using 4N HCl. Heating may be necessary. Water is then used to dilute the sample to an appropriate volume.

Example E: Preparation of Gd-159

Gadolinium-159 is prepared by sealing gadolinium oxide (1.1 mg) in a quartz vial. The vial is welded inside an aluminum can and irradiated for 30 hours in a reactor at a neutron flux of 8×10^{13}

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neutron/cm².sec. The contents of the quartz vial is dissolved using HCl. Water is added to obtain a solution of Gd-159 in 0.1N HCl.

5 Example F: Preparation of Y-90

A non-radioactive Ytterium (Y) solution was prepared by dissolving 15.1 mg of YCl₃·6H₂O in 11.24 mL of water. A quantity of 1500 µL of this solution was added to a vial containing 0.5 mL of Y-90 solution (prepared by neutron irradiation of 1 mg of Y₂O₃ followed by dissolution in 1N HCl to give a final volume of 0.5 mL).

15 Example G: Preparation of Yb-175 and Lu-177

When the procedure of Examples C, D, E or F are repeated using the appropriate oxide, the radioisotopes of Ytterbium-175 (Yb-175) and Lutetium-177 (Lu-177) are prepared.

20 Preparation of Final Products

Example 1: Preparation and Biodistribution of
Sm-DOTMP and Sm-153-DOTMP

25 The ligand of Example A (22 mg) was dissolved in 878 µl of distilled water and 15 µl of 50 percent NaOH. A volume of 15 µl of this solution was transferred to a vial containing 1.5 mL of Sm solution (0.3 mM Sm in 0.1N HCl spiked with 2 µl of Sm-153 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Sm found as a complex was >99 percent as determined by ion exchange chromatography. This yielded a solution containing Sm at 0.3 mM with a ligand to metal molar ratio of approximately 1.5.

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Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μ L of the Sm solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection.

5. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 μ L standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table I. The numbers represent the average of 3 rats per data point.

TABLE I
% INJECTED DOSE IN SEVERAL
TISSUES FOR Sm-DOTMP[†]

Tissue	% Dose
Bone	58.1
Liver	0.06
Kidney	0.27
Spleen	0.004
Muscle	0.15
Blood	0.004

[†] Ligand to Sm Molar Ratio of approximately 1.5

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Example 2: Preparation and Biodistribution of
Ho-DOTMP and Ho-166-DOTMP

5 The ligand of Example A (22 mg) was dissolved
in 878 μ L of distilled water and 15 μ L of 50 percent
NaOH. A volume of 30 μ L of this solution was
transferred to a vial containing 1.5 mL of Ho solution
(0.6 mM Ho in 0.1N HCl spiked with 2 μ L of Ho-166
tracer). The pH was adjusted to 7-8 using NaOH and the
10 amount of Ho found as a complex was greater than 99
percent as determined by ion exchange chromatography.
This yielded a solution containing 0.6 mM Ho with a
ligand to metal molar ratio of approximately 1.5.

15 Sprague Dawley rats were allowed to acclimate
for five days then injected with 100 μ L of the Ho
solution described above via a tail vein. The rats
weighed between 150 and 200 g at the time of injection.
After 2 hours the rats were killed by cervical
20 dislocation and dissected. The amount of radioactivity
in each tissue was determined by counting in a NaI
scintillation counter coupled to a multichannel
analyzer. The counts were compared to the counts in 100
25 μ L standards in order to determine the percentage of the
dose in each tissue or organ. The percent of the
injected dose in several tissues are given in Table II.
The numbers represent the average of 3 rats per data
point.

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TABLE II
% INJECTED DOSE IN SEVERAL
TISSUES FOR Ho-DOTMP¹

Tissue	% Dose
Bone	57
Liver	0.07
Kidney	0.4
Spleen	0.006
Muscle	0.3
Blood	0.07

¹ Ligand to Ho Molar Ratio of
approximately 1.5

Example 3: Preparation and Biodistribution of

Sm-DOTMP, Sm-153-DOTMP, Ho-DOTMP and
Ho-166-DOTMP

A quantity of 14.5 mg of the ligand of Example B was placed in a vial and dissolved in 760 μ L of water and 5 μ L of 50 percent NaOH. A volume of 1100 μ L of Sm solution (0.3 mM Sm in 0.1N HCl) which was spiked with Sm-153, was placed in a separate vial and 10 μ L of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 3 plastic columns containing 1.5 mL of cation exchange resin (Sephadex™ C-25 from Pharmacia). The amount of Sm as a complex was determined to be 99 percent by cation exchange chromatography.

A volume of 1100 μ L of Ho solution (0.6 mM Ho in 0.1N HCl) which was spiked with Ho-166, was placed in a separate vial and 20 μ L of the above ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 2 plastic columns containing 1.5 mL of cation exchange resin (Sephadex C-25 from Pharmacia). The amount of Ho as a

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complex was determined to be 99 percent by cation exchange chromatography.

5 Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μ L of the solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of
10 radioactivity determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 μ L standards in order to determine the percentage of the dose in each tissue or organ. The
15 percent of the injected dose in several tissues are given in Table III. The numbers represent the average of 3 rats per data point.

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TABLE III
% INJECTED DOSE IN SEVERAL TISSUES FOR
DOTMP METAL COMPLEXES

25

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Tissue	Sm	Ho
Bone	50	64
Liver	0.37	0.19
Kidney	0.29	0.32
Spleen	0.04	0.05
Muscle	0.49	0.22
Blood	0.12	0.17

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Example 4: Preparation and Biodistribution of
Gd-DOTMP and Gd-159-DOTMP

5 The ligand of Example B (14.5 mg) was placed in
a vial and dissolved in 760 μ L of water and 5 μ L of 50
percent NaOH. A volume of 1000 μ L of Gd solution (0.3
mM Gd in 0.1N HCl) which contained tracer quantities of
Gd-159, was placed in a separate vial and 15 μ L of the
ligand solution was added. The pH of the solution was
10 adjusted to 7-8 using NaOH and the amount of Gd as a
complex was determined to be >99 percent by cation
exchange chromatography.

15 A Sprague Dawley rat was allowed to acclimate
for five days then injected with 175 μ L of the solution
described above via a tail vein. The rat weighed 155 g
at the time of injection. After 2 hours the rat was
killed by cervical dislocation and dissected. The
amount of radioactivity in each tissue was determined by
20 counting in a NaI scintillation counter coupled to a
multichannel analyzer. The counts in each tissue were
compared to the counts in 175 μ L standards in order to
determine the percentage of the dose in each tissue or
25 organ. The percent of the injected dose in several
tissues are given in Table IV.

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TABLE IV
% INJECTED DOSE IN SEVERAL TISSUES
FOR Gd-DOTMP¹

Tissue	% Dose
Bone	50
Liver	0.08
Kidney	0.25
Spleen	None Detected*
Muscle	0.08
Blood	0.06

¹ Ligand to Gd molar ratio of approximately 1.5

*counts in the spleen were below background background

Example 5: Preparation and Biodistribution of
Lu-DOTMP and Lu-177-DOTMP

The ligand of Example B (15.8 mg) was dissolved in 963 μ L of distilled water and 8 μ L of 50 percent NaOH. A volume of 15 μ L of this solution was transferred to a vial containing 1.5 mL of Lu solution (0.3 mM Lu in 0.1N HCl spiked with 2 μ L of Lu-177 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Lu found as a complex was >99 percent by ion exchange chromatography. This yielded a solution containing 0.3 mM Lu with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μ L of the Lu solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI

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scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 μ L standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table V. The numbers represent the average of 3 rats per data point.

TABLE V
% INJECTED DOSE IN SEVERAL TISSUES
FOR Lu-DOTMP¹

Tissue	% Dose
Bone	54
Liver	0.08
Kidney	0.3
Spleen	0.006
Muscle	0.04
Blood	0.09

¹ Ligand to Lu molar ratio of approximately 1.5

Example 6: Preparation and Biodistribution of
Y-DOTMP and Y-90-DOTMP

To the solution of Y and Y-90 prepared in Example F was added 200 μ L (0.0266 moles) of DOTMP from Example B in water and the pH of the solution adjusted to 7.5 using 50 percent NaOH and 1N NaOH. The percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 1.7.

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Sprague Dawley rats were allowed to acclimate for eight days then injected with 150 μ L of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150 μ L standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VI. The numbers represent the average of 5 rats per data point.

TABLE VI
% INJECTED DOSE IN SEVERAL TISSUES
FOR Y-DOTMP¹

Tissue	% Dose
Bone	33
Liver	0.06
Kidney	0.35
Spleen	0.01
Muscle	0.31
Blood	0.12

¹ Ligand to Y molar ratio of approximately 1.7

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Example W (Comparative)

To a vial containing 0.5 mL of Y-90 solution (prepared by the irradiation of 1 mg of Y_2O_3 followed by dissolution in 1.1N HCl to give a final volume of 0.5 mL) was added 1.5 mL of water to give a 8.86×10^{-3} molar solution of Y containing tracer Y-90. To 2 mL (1.772×10^{-5} mole) of this solution was added 133 μ L (1.676×10^{-4} mole) of 1.26M ethylenediaminetetra-
methylenephosphonic acid (EDTMP) solution where upon the solution became turbid. The solution cleared up upon addition of 50 μ L of 50 percent NaOH. To this solution was added 40 μ L (5.04×10^{-5} mole) more of 1.26M EDTMP solution. The pH of the resulting solution was 7.5 and the percent of the Y as a complex was determined by cation exchange chromatography to be >99 percent. This yielded a solution with a ligand to metal molar ratio of approximately 123.

Sprague Dawley rats were allowed to acclimate for eight days then injected with 150 μ L of the Y solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 150 μ L standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table W. The numbers represent the average of 5 rats per data point.

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TABLE W
% INJECTED DOSE IN SEVERAL TISSUES
FOR Y-EDTMP¹

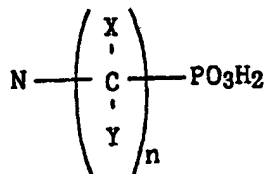
Tissue	% Dose
Bone	30
Liver	0.09
Kidney	0.30
Spleen	0.01
Muscle	0.58
Blood	0.15

¹ Ligand to Y molar ratio of
approximately 123

15 (There are no Examples X and Y.)

Example Z (Comparative)

20 In a method similar to that previously used,
compositions were prepared containing complexes of Sm-
153 with several commercially available phosphonic
acids which do not contain the alkylene linkage
between the nitrogen and the phosphorus atoms (which
25 linkage is required in the present ligand).



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The two hour biolocalization of Sm-153 in rats for these compositions was determined as previously described. The results are given in Table X. The ligands used include methylenediphosphonic acid (MDP) and hydroxyethylidinediphosphonic acid (HEDP) which contain a P-CH₂-PO₃H₂ and a P-C(CH₃)(OH)-PO₃H₂ linkage, respectively; pyrophosphate (PYP) which contains a P-O-PO₃H₂ linkage; and imidodiphosphate (IDP) which contains a N-PO₃H₂ linkage. Metal complexes of these ligands are known skeletal agents. For example, Tc complexes of MDP, HEDP, and PYP have been used commercially as diagnostic bone agents. However, these ligands were inadequate for selectively delivering Sm-153 to the skeletal system as exemplified by the large fraction of the radioactivity found in the liver and/or blood.

Table Z shows the biolocalization of Sm-153 in rats two hours after injection and the results represent the percent of injected dose in tissue.

TABLE Z

% Dose In	Sm-153 MDP	Sm-153 HEDP	Sm-153 PYP	Sm-153 IDP
Bone	2	21	2	0.6
Liver	85	3.5	73	36
Blood	0.23	13	0.23	0.04

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The numbers given in Table Z for Sm-153-MDP, Sm-153-HEDP, Sm-153-PYP and Sm-153-IDP represent the average of the results of five, five, three and three rats, respectively.

5

Example 7: Preparation of Sm-DOTMP or Ho-DOTMP Kit
Using HEPES Buffer

A 0.1M solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma™ Chemical Co., St. Louis, MO) at a pH of 7.43 was prepared. A 0.0066M solution of DOTMP was prepared by dissolving 68.2 mg (1.084×10^{-4} μ mole) of DOTMP in 16.4285 mL of 1N NaOH. Into each of seven 10 mL serum vials was placed 0.600 mL (3.96 μ mole) of DOTMP solution and 3.00 mL of 0.1M HEPES buffer solution. Each serum vial was then placed in a dry ice/acetone bath until the liquid was frozen and then placed in a Virtis Freeze Dryer Apparatus overnight which gave the aqueous components as a dry white powder in the bottom of the serum vials. The serum vials were then stoppered and sealed by crimping. These kits were formulated to receive 6 mL of either SmCl_3 (3×10^{-4} mole) or HoCl_3 (6×10^{-4} mole) in 0.1N HCl.

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Example 8: Reconstitution of Sm-DOTMP or Ho-DOTMP Kit
Containing HEPES Buffer

A 6.0 mL addition of SmCl_3 (3×10^{-4} M spiked with Sm-153 in 0.1N HCl) was made to one of the kits described in Example 7. The pH of the resulting reconstituted kit was 7.5 and the percent of Sm that was complexed was determined using cation exchange chromatography to be >99 percent.

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Similarly, a 6.0 mL addition of HoCl_3 (6×10^{-4} M spiked with Ho-166) in 0.1N HCl was made to

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one of the kits described in Example 7. The pH of the resulting solution was 7.5 and the percent of Ho that was complexed was determined using cation exchange chromatography to be >97 percent.

5

Example 9: Reconstitution and Biodistribution of
Sm-HEPES-DOTMP Kits

10 A kit from Example 8 was treated with 6.0 mL of SmCl_3 ($3 \times 10^{-4}\text{M}$ spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was 7.5 and the percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

15

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μL of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 100 μL standards in order to
25 determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VII. The numbers represent the average of 3 rats per data point.

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TABLE VII
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP/HEPES BUFFER

Tissue	% Dose
Bone	58
Liver	0.06
Kidney	0.29
Spleen	0.01
Muscle	0.18
Blood	0.06

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10
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Example 10: Preparation of Sm-DOTMP Kits
Using Bicarbonate Buffer

A 0.009M solution of DOTMP at pH 6.66 was prepared by adding 141.5 mg (2.25×10^{-4} mole) of DOTMP to 9 mL of 1N NaOH and diluting to 25 mL final volume. A 0.4M solution of sodium bicarbonate (NaHCO_3) was prepared by dissolving 8.4 g of NaHCO_3 in 250 mL of water. Kits were prepared by adding 3.0 mL of NaHCO_3 solution and 0.300 mL of DOTMP solution to each of seven 10 mL serum vials and treating them as described in Example 7 to give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of SmCl_3 ($3 \times 10^{-4}\text{M}$) in 0.1N HCl which would give a ligand to metal ratio of 1.5:1.

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25
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Example 11: Reconstitution and Biodistribution of
Sm-DOTMP Kits Using Bicarbonate Buffer

A kit from Example 10 was treated with 6.0 mL of SmCl_3 ($3 \times 10^{-4}\text{M}$ spiked with Sm-153) in 0.1N HCl.

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The pH of the resulting solution was 6.55 and was adjusted to 7.27 by the addition of 60 μ L of 1N NaOH. The percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

- 5 Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μ L of the Sm solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection.
- 10 After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were
- 15 compared to the counts in 100 μ L standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VIII. The numbers
- 20 represent the average of 3 rats per data point.

TABLE VIII
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP¹/BICARBONATE

Tissue	% Dose
Bone	65
Liver	0.07
Kidney	0.34
Spleen	0.01
Muscle	0.30
Blood	0.04

¹Ligand to Sm molar ratio of approximately 1.5

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Example 12: Preparation of DOTMP Kit
Using Excess Base

5 A 0.009M solution of DOTMP was prepared as described in Example 10 except more NaOH was added such that the final solution was pH 10.66. Kits were prepared by adding 0.300 mL of DOTMP solution and 0.700 mL of 1.0N NaOH solution to each of five 10 mL serum vials and treating them as described in Example 7 to
10 give the final kit containing a white dry solid. These kits were formulated to receive 6.0 mL of SmCl_3 ($3 \times 10^{-4}\text{M}$) in 0.1N HCl which would give a ligand to metal ratio of 1.5:1.

15 Example 13: Reconstitution and Biodistribution of
DOTMP Kits Using Excess Base and
Phosphate Buffer

A kit from Example 12 was treated with 5.4 mL
20 of SmCl_3 ($3 \times 10^{-4}\text{M}$ spiked with Sm-153) in 0.1N HCl and 0.6 mL of SmCl_3 ($3 \times 10^{-4}\text{M}$ spiked with Sm-153) in 0.1N HCl. The pH of the resulting solution was between 10 and 11. The pH was adjusted to 7.79 by the addition of 0.200 mL of 1.05M phosphate buffer (pH 7.49). The
25 percent of the Sm as a complex was determined using cation exchange chromatography to be >99 percent.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 μL of the Sm
30 solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount
of radioactivity in each tissue was determined by
35 counting in a NaI scintillation counter coupled to a

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multichannel analyzer. The counts in each tissue were compared to the counts in 100 μ L standards in order to determine the percentage of the injected dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IX. The numbers represent the average of 5 rats per data point.

TABLE IX
% INJECTED DOSE IN SEVERAL TISSUES
FOR Sm-DOTMP1/PHOSPHATE

Tissue	% Dose
Bone	59
Liver	0.85
Kidney	0.41
Spleen	0.03
Muscle	0.35
Blood	0.11

Ligand to Sm molar ratio of
approximately 1.5

Example 14: Preparation of 18 mL Ho-DOTMP Kits

A 0.009M solution of DOTMP at pH 6.66 was prepared as described in Example 10 except more NaOH was added such that the final solution was at pH 10.19. Kits were prepared by adding 1.800 mL of DOTMP solution and 2.100 mL of 1N NaOH solution to each of twelve 20 mL serum vials. These vials were then treated as described in Example 7 to give the final kits containing a white, dry solid. These kits were formulated to receive 18.0 mL of HoCl_3 ($6 \times 10^{-4}\text{M}$) which would give a ligand to metal ratio of 1.5:1.

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Example 15: Reconstitution and Biodistribution of
18 mL Ho-DOTMP Kits

5 A kit from Example 14 was treated with 18.0 mL
of HoCl_3 ($6 \times 10^{-4}\text{M}$ spiked with Ho-166) in 0.1N HCl.
The solution was then treated with 0.6 mL of 1.05M
phosphate buffer (pH 7.49) which brought the pH down to
7.53. The percent of the Sm as a complex was determined
using cation exchange chromatography to be >99 percent.

10 Sprague Dawley rats were allowed to acclimate
for five days then injected with 100 μL of the Sm
solutions described above via a tail vein. The rats
weighed between 150 and 200 g at the time of injection.
15 After 2 hours the rats were killed by cervical
dislocation. Tissues were taken, weighed and the amount
of radioactivity in each tissue was determined by
counting in a NaI scintillation counter coupled to a
multichannel analyzer. The counts in each tissue were
20 compared to the counts in 100 μL standards in order to
determine the percentage of the injected dose in each
tissue or organ. The percent of the injected dose in
several tissues are given in Table X. The numbers
25 represent the average of 5 rats per data point.

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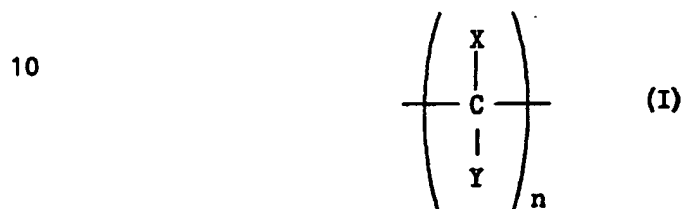
TABLE X
% INJECTED DOSE IN SEVERAL TISSUES
FOR Ho-DOTMP¹/PHOSPHATE

Tissue	% Dose
Bone	60
Liver	0.12
Kidney	0.35
Spleen	0.08
Muscle	0.21
Blood	0.04

¹Ligand to Ho molar ratio of
approximately 1.5

CLAIMS:

1. A composition which comprises a complex having (1) a macrocyclic aminophosphonic acid, containing 1,4,7,10-tetraazacyclododecane as the macrocyclic moiety, or a physiologically acceptable salt thereof, wherein the nitrogen and phosphorous are interconnected by an alkylene or substituted alkylene radical of the formula



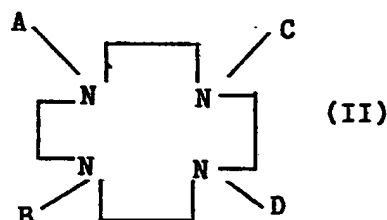
- 15 wherein: X and Y are independently hydrogen, hydroxyl, carboxyl, phosphonic, or hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals; and n is 1-3, with the
- 20 proviso that when $n > 1$, each X and Y may be the same as or different from the X and Y of any other carbon atom, and (2) at least one radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175, and
- 25

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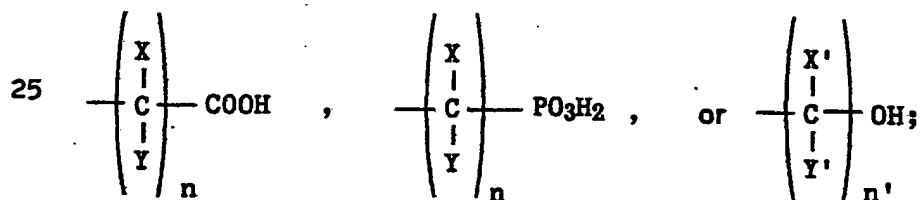
wherein the resulting composition is therapeutically effective.

2. The composition of Claim 1 wherein X and Y are hydrogen and n is 1.

3. The composition of Claim 1 wherein the macrocyclic aminophosphonic acid has the structure



wherein: substituents A, B, C and D are independently hydrogen, hydrocarbon radicals having from 1-8 carbon atoms, or a moiety of the formula



30 and physiologically acceptable salts of the acid radicals, wherein: X, Y and n are as defined in Claim 1; X' and Y' are independently hydrogen, methyl or ethyl radicals; n' is 2 or 3, with the proviso that at least

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two of said nitrogen substituents is a phosphorus-containing group.

5 4. The composition of Claim 3 wherein the macrocyclic aminophosphonic acid is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt.

10 5. The composition of any one of the preceding claims wherein the radionuclide is Gd-159.

6. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Sm-153.

15 7. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Lu-177.

8. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Yb-175.

20 9. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Ho-166.

10. The composition of Claim 1, 2, 3 or 4 wherein the radionuclide is Y-90.

25 11. A sterile composition suitable for administration to an animal wherein the composition contains a complex as claimed in any one of the preceding claims and wherein the radionuclide in dosage form is present in an amount containing at least 0.02 mCi per kilogram of body weight of said animal.

30 12. The composition of Claim 11 wherein the radionuclide in dosage form is present in an amount

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containing at least 0.2 mCi per kilogram of body weight of said animal.

5 13. The composition of any one of the preceding claims wherein the ligand to radionuclide molar ratio is at least about 1:1.

 14. The composition of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 3:1.

10 15. The composition of Claim 13 wherein the ligand to radionuclide molar ratio is from 1:1 to 1.5:1.

 16. A pharmaceutical formulation which comprises the composition as claimed in any one of the preceding claims and a pharmaceutically acceptable carrier.

 17. A pharmaceutical formulation of Claim 16 wherein the formulation having the complex and a buffer present are frozen in a kit form, and which frozen formulation is later thawed prior to use.

25 18. A method for the therapeutic treatment of an animal having one or more calcific tumors which comprises administering to said animal a therapeutically effective amount of at least one pharmaceutical formulation as claimed in Claim 16 or 17 or of at least one composition as claimed in Claims 1 to 15.

30 19. A method for the therapeutic treatment of an animal having bone pain which comprises administering to said animal a therapeutically effective amount of at least one pharmaceutical formulation as claimed in Claim 16 or 17 or of at least one composition as claimed in
35 Claims 1 to 15.

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20. The method of Claim 18 or 19 wherein the animal is a human.

5 21. A process for preparing a composition as claimed in Claim 1 which comprises reacting a radionuclide of Sm-153, Gd-159, Ho-166, Lu-177, Y-90 or Yb-175 with the macrocyclic aminophosphonic acid as claimed in any one of the preceding claims, in water at a controlled pH.

10 22. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Sm-153,
15 in water at a controlled pH.

23. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic
20 acid or a physiologically acceptable salt with Gd-159, in water at a controlled pH.

24. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic
25 acid or a physiologically acceptable salt with Ho-166, in water at a controlled pH.

25. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic
30 acid or a physiologically acceptable salt with Lu-177, in water at a controlled pH.

35 26. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-

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tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Y-90, in water at a controlled pH.

5 27. The process of Claim 21 for preparing a composition which comprises reacting 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid or a physiologically acceptable salt with Yb-175, in water at a controlled pH.

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AMENDED CLAIMS

[received by the International Bureau
on 16 May 1990 (16.05.90);
original claims 18 and 19 amended; other claims
unchanged (1 page)]

containing at least 0.2 mCi per kilogram of body weight
of said animal.

5 13. The composition of any one of the preceding
claims wherein the ligand to radionuclide molar ratio is
at least about 1:1.

14. The composition of Claim 13 wherein the
ligand to radionuclide molar ratio is from 1:1 to 3:1.

10 15. The composition of Claim 13 wherein the
ligand to radionuclide molar ratio is from 1:1 to 1.5:1.

15 16. A pharmaceutical formulation which
comprises the composition as claimed in any one of the
preceding claims and a pharmaceutically acceptable
carrier.

20 17. A pharmaceutical formulation of Claim 16
wherein the formulation having the complex and a buffer
present are frozen in a kit form, and which frozen
formulation is later thawed prior to use.

25 18. A method for the therapeutic treatment of
an animal having one or more calcific tumors which
comprises administering to said animal a therapeutically
effective amount of at least one pharmaceutical
formulation as claimed in Claim 16.

30 19. A method for the therapeutic treatment of
an animal having bone pain which comprises administering
to said animal a therapeutically effective amount of at
least one pharmaceutical formulation as claimed in Claim
16.

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INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US89/05782**

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *	
According to International Patent Classification (IPC) or to both National Classification and IPC	
IPC (5): A 61 K 43700, 49/02	
II. FIELDS SEARCHED	
Minimum Documentation Searched ⁷	
Classification System	Classification Symbols
U.S.	424/1.1., 424/9, 534/10
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *	

STN MESSENGER Structure Search

III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP, A, 287,465 (GUERBET S.A.) 19 OCTOBER 1988. See the entire document.	1-10
A	EP, A, 0,164,843 (THE DOW CHEMICAL COMPANY) 18 DECEMBER 1985, see the entire document.	18-20
Y P	US, A, 4,885,363 (TWEEDLE et al.) 05 DECEMBER 1989. See the entire document.	1-27
P	US, A, 4,882,142 (SIMON et al.) 21 NOVEMBER 1989. See the abstract.	1-17,22-27
A,P	US, a, 4,853,209 (KAPLAN et al.) 01 AUGUST 1989. See the abstract.	
Y	US, A, 3,965,254 (TOFE et al.) 22 JUNE 1976 See the abstract	18-20
Y	US, A, 4,017,595 (SUBRAMANIAN et al.) 12 APRIL 1977 See column 1, line 5 bridging column 2, line 10.	18-20
Y	US, A, 4,187,284 (ROLLESTON et al.) 05 FEBRUARY 1980 See column 1, lines 23-54.	18-20

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

15 FEBRUARY 1990

International Searching Authority

ISA/US

Date of Mailing of this International Search Report

22 MAR 1990

Signature of Authorized Officer

John M. Covert
JOHN M. COVERT

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Int. J. Applied Radiation and Isotopes, Volume 14, issued 1963 (Northern Ireland) ROSOFF et al., "Distribution and Excretion of Radioactive Rare-Earth Compounds in Mice", see p. 132, second column bridging column 3, first column; see page 134, bottom half, first column.	18-20
Y	Chemical Abstracts, Volume 87, issued 1977 (Columbus, Ohio, USA) G. Subramanian et al., "Indium-113m labeled polyfunctional phosphonates as bone imaging agents", abstract No. 179938h, Nucl.-Med. (Stuttgart) Suppl., 1977, 14, 671-8 (Eng).	18-20

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter^{1,2} not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out^{1,3}, specifically:

3. ☒ Claim numbers 18-20, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.